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Crypt fission in the small intestine of the rat

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There is general agreement that new intestinal crypts of the small and large intestine develop by a process of longitudinal fission from the bases of existing ones (Clarke, 1972; Cairnie & Millen, 1975; Maskens, 1978; Tseng *et al.*, 1978; Maskens & Dujardins-Loits, 1981; Potten, 1983; Bristol & Williamson, 1984; Cheng & Bjerknes, 1985; St. Clair & Osborne, 1985). The frequency of this process is high in very young animals and declines rapidly with age (Maskens, 1978; Maskens & Dujardin-Loits, 1981; Cheng & Bjerknes, 1985; St. Clair & Osborne, 1985). In response to radiation (Cairnie & Millen, 1975; Tseng *et al.*, 1978; Khokhar & Potten, 1979) or chemical (Deschner, 1980; St. Clair, 1985) injury, the percentage of crypts in fission (PCF) increases markedly and subsequently declines as crypt number is restored. Changes in crypt cell proliferation rate and total crypt number (Cairnie & Millen, 1975) after radiation inury and during the recovery process have been noted. However, only one study has documented PCF as a function of radiation dose and time after irradiation. Possible interrelationships among PCF, crypt cell proliferation, crypt number, and crypt density during recovery from radiation injury need to be established. This investigation was an attempt to establish such relationships.

Materials and methods

X-irradiation

Young adult rats were anesthetized with Nembutal ($40\,mg\,kg^{-1}$) and the intestine from the ligament of Treitz to the ileo-caecal junction temporarily exteriorized through a midline incision. The body of the rat was shielded by 3.2 mm of lead while the exteriorized loop of bowel received 2.5, 5.0, 10.0, or 13.5 Gy of 250 kVp X-rays. Samples of irradiated intestine as well as unirradiated duodenum and transverse colon were obtained 7–63 days later.

PCF, DPM/crypt, crypts per intestine, and crypt density

Samples of intestine for all measurements but crypt density were processed and values calculated according to the methods of St. Clair and Osborne (1985).

Estimation of crypt density was made as follows. The entire ileum and jejunum was removed and flushed with cold saline. The length was then measured and the intestine laid flat with the distal end clamped at the ileo-caecal valve. The tip of a glass pipette, held vertically with respect to the long axis of the gut, was then inserted into the open end with the aid of plastic tubing and held securely. The volume of saline which was required to achieve a hydrostatic pressure of 25 cm of saline was measured. Using the assumption that the lumen of the gut was a cylinder, the measured length (l) and volume were used to calculate the radius (r). The intestinal length and radius were then used in the equation $A = 2\pi rl$ to calculate the intestinal surface area. Crypts cm^{-2} were then calculated.

In Series I with a range of X-ray doses, samples were taken from 7–35 days post-irradiation. In Series II with a single dose of 10.0 Gy, samples were taken 7–63 days later. All animals utilized for dpm/crypt data were injected i.p. 1 h before sacrifice with $1\,\mu Ci\,g^{-1}$ methyl[^{3}H]-thymidine.

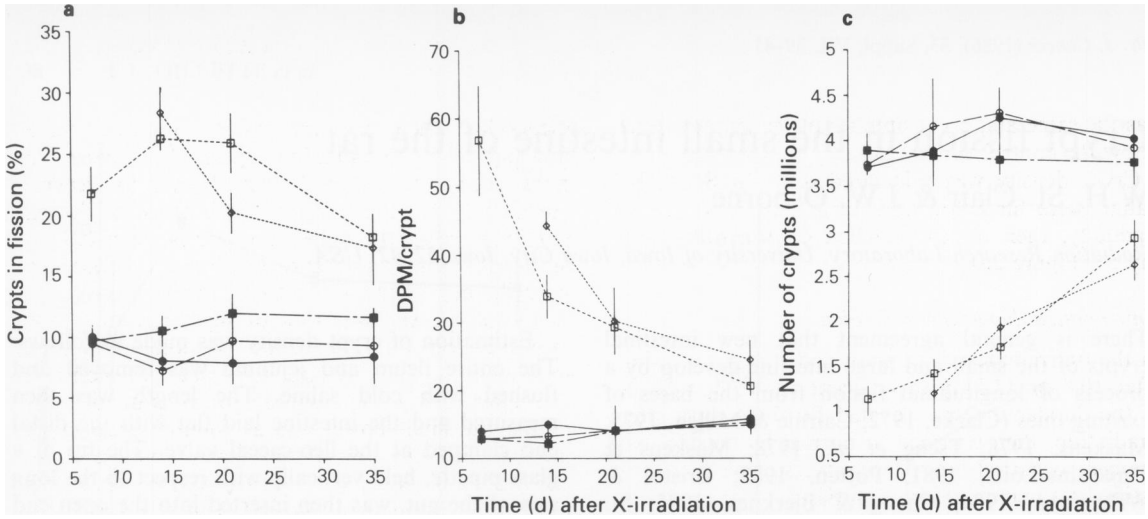
Results

Irradiated jejunum and ileum

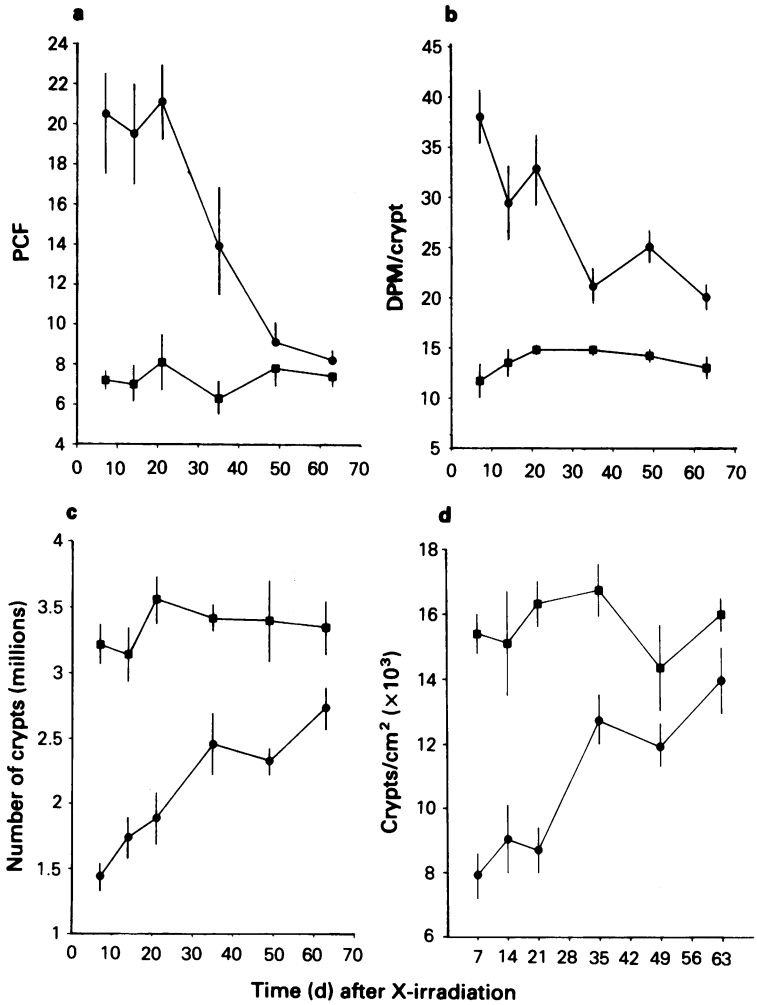
In Series I, PCF was unchanged after 2.5 or 5.0 Gy. It increased markedly after 10.0 or 13.5 Gy and peaked at a value four-fold higher than control at 14 days post-irradiation. PCF then decreased gradually but did not drop to control values by day 35 (Figure 1a). In the Series II study, the PCF was significantly elevated by 7 days after X-irradiation, peaked at 21 days, and declined toward control values which were reached after 49 days (Figure 2a).

In Series I, DPM/crypt was substantially elevated above control only after 10.0 Gy and 13.5 Gy. The values were greatest at 7 days and declined throughout 35 days without returning to normal (Figure 1b). The pattern in Series II was similar (Figure 2b).

In Series I, the total number of crypts in the combined jejunum and ileum did not change significantly after 2.5 or 5.0 Gy, but was markedly decreased after 10.0 or 13.5 Gy at 7 days post-irradiation. This was followed by a gradual rise with time, but control values were not reached within 35 days (Figure 1c). In Series II, the trend



Figures 1a–c Percentage of crypts in fission (a), DPM per crypt (b), and number of crypts in the combined jejunum and ileum (c) after 2.5 (○); 5.0 (■); 10.0 (□); or 13.5 (◇) Gy of X-rays to only the exteriorized intestine. Mean ±2 s.e. $n \geq 5$. (●) = control.



Figures 2a–d Percentage of crypts in fission (a), DPM per crypt (b), number of crypts (c), and crypt density (d) in the combined jejunum and ileum, after 10.0 Gy of X-rays to only the exteriorized intestine. Mean ±2 s.e. $n \geq 5$. (■) control; (●) 10 Gy.

was the same and crypt number plateaued at a level below the control (Figure 2c).

Crypt density was calculated only for Series II. Values were far below control at day 7, recovered gradually, then almost reached control values by day 63 (Figure 2d).

Unirradiated duodenum and colon

In the unirradiated duodenum and transverse colon after 10.0 or 13.5 Gy to adjacent tissue, dpm/crypt in the duodenum was elevated for 7–14 days after irradiation, then returned to normal by day 21. No changes occurred in the transverse colon. Crypt fission was unchanged in each tissue.

Discussion

In the Series I, 10 Gy to the small bowel was the threshold X-ray dose needed to produce a significant influence on PCF, crypt cell proliferation (CCP), and intestinal crypt number (ICN). Generally, when ICN was rising after reaching a low point due to radiation injury, PCF and CCP were declining. The PCF results are in general agreement with those found by Khokhar & Potten (1979). It seems the system recognizes when ICN is approaching normality and therefore has a lessened need for crypt fission and CCP. In our laboratory, this same pattern was seen during recovery of the intestine from an IP injection of mechlorethamine hydrochloride or cyclophosphamide. In contrast (St. Clair, 1985), the response to resection of 70% of the rat small bowel was as follows: total crypt number in the remaining ileum and jejunum showed no tendency to return to normal, CCP was increased, and PCF unchanged. These findings plus the decline in PCF with increasing age of postnatal rats suggests that *crypt density* rather than total crypt number modulates PCF.

Since there was a change in CCP of unirradiated duodenum, but no change in PCF, there is the suggestion that humoral factors are related to CCP, but that local factors regulate PCF.

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